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The Influence of Phenobarbital, Diphenylhydantoin, Trimethadione and Ethosuximide on Rat Liver Demethylase Activity

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THE INFLUENCE OF PHENOBARBITAL, DIPHENYLHYDANTOIN, TRIMETHADIONE AND ETHOSUXIMIDE ON RAT LIVER DEMETHYLASE ACTIVITY

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

With the continual increase in the number and types of chemical entities available for drug therapy, a corresponding increase in interest regarding the mechanisms by which these agents are inactivated has developed. This topic is a main concern of current literature. In selecting drug therapy for one of the present drug treatable diseases, a clinician will generally be able to select from more than one family or group of drugs. On occasion, it may be desirable to use more than one drug which may or may not be of the same chemical family or group. In any case, the importance of locating the site of inactivation is increasing in stature. A question that might arise is whether or not the concurrent use of two or more types of agents would compete for the same site of inactivation. It would seem that before this could be determined, the location and the mechanism of inactivation for each group or family of drug agents involved should be determined.

Recently interest has been directed towards other systems that might be affected in addition to those for which the drug was administered. Because the liver is the main detoxifying organ, emphasis has been placed on the alteration of microsomal enzyme activity in this tissue. Some of the early work in this area was performed by Conney et al. (1) who evaluated rat liver enzyme induction caused by phenobarbital. In this work these investigators found that injection of phenobarbital caused a several-fold increase in the microsomal enzyme activity of young rats. A considerable increase in activity

was demonstrated within a 24-hour period following the administration of the drug. In addition to the increase in enzyme activity, the drug also caused an increase in the size of the livers. This effect is attributed to increased enzyme formation resulting in a higher protein content of the treated livers.

Orrenius et al. (2) state that prolonged phenobarbital treatment leads to an increase in both liver weight and microsomal protein per unit of liver weight. Both values returned to normal within two weeks following the discontinuance of phenobarbital.

The studies of Arias et al. (3) indicate that one-half of the rat liver proteins turn over within 2.5 days. The evidence obtained by the use of electron microscopy indicates that synthesis of microsomal enzymes occurs primarily in smooth endoplasmic reticulum.

The findings of Holtzman and Gillette (4) led them to conclude that stimulation of protein production was responsible for the increase in endoplasmic reticulum components following phenobarbital administration. These results supported earlier work of a similar nature by Conney and Gilman (5), and Orrenius et al. (6). It has become generally accepted that elevated levels of microsomal enzyme activity following phenobarbital administration is due to increased synthesis of protein and is not the result of decreased protein breakdown.

Many drug products are dealkylated by liver enzymes. An example is the demethylation of aminopyrine by microsomal demethylase. This enzyme has become a valuable investigational tool for the measurement

of enzyme activity alterations caused by drug administration. The removed methyl groups from aminopyrine can be converted to formaldehyde which can be readily assayed (7).

Eling et al. (8) noted that the intraperitoneal administration of diphenylhydantoin causes an increase in the hepatic metabolism of hexobarbital, benzphetamine and aminopyrine in neonatal rats. Diphenylhydantoin increased liver weight but did not change the concentration of protein in the liver tissue. These investigators also demonstrated the metabolism of aminopyrine and benzphetamine in adult rats. A previous report of Platt and Cockrill (9) indicated somewhat different results. They did not observe a diphenylhydantoin-induced alteration in liver size nor increase in the metabolism of aminopyrine by microsomal enzymes. A possible factor in these results may be that the rats of Platt and Cockrill were given diphenylhydantoin orally which possibly did not result in blood levels of the drug sufficiently high to initiate enzyme induction.

Anti-epileptic agents are occasionally used concurrently to achieve better control of the disease (10). The effect that each agent has on microsomal enzyme activity could have considerable bearing on the dosage and the frequency with which the drug is to be administered. The metabolic degradation of diphenylhydantoin can be enhanced by the simultaneous administration of phenobarbital and certain other drugs (11). Since both phenobarbital and diphenylhydantoin are occasionally used together in treatment of the epileptic, dosage adjustments must be made to achieve an optimal maintenance regimen. This type of drug interaction might also suggest close scrutiny of medicaments that

might be administered for other purposes to patients on phenobarbital, diphenylhydantoin or any other agents. Phenobarbital is not the only agent that is known to alter the drug metabolism because of its influence on microsomal activity; the list is long and diverse including such chemicals as 3-methylcholanthrene, 3,4-benzpyrene and chlordane (8).

The study of the effects of phenobarbital on liver microsomal enzyme activity has been rather extensive (1-6,11). As a result phenobarbital appears to be a "yardstick" by which many enzyme induction studies are compared. Although some work has been done in this area using diphenylhydantoin, its study has not been nearly so extensive.

Of the many anti-epileptic agents available, the most commonly used compounds can be divided into four categories: the barbiturates and related drugs, the hydantoins, the oxazolidines and the succinimides (12). Because of the possibility of concurrent use of the compounds in the treatment of epilepsy, it would be advantageous to have some information concerning the effect that each group has individually on microsomal enzyme systems before therapy is instituted. This information would enable the clinician to more adequately predict drug response thus reducing dosage regimen adjusting time and also possibly reducing the incidence of adverse reactions or interactions. The effect of both phenobarbital and diphenylhydantoin on microsomal enzyme activity has been previously explored and the objective of this study was to investigate what effects the administration of

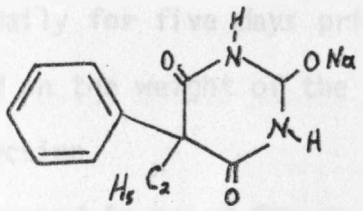
trimethadione and ethosuximide would have under similar circumstances. Little or no information has been published on the influence of the latter two compounds on demethylase activity.

For this study the barbiturates were represented by phenobarbital, the hydantoins by diphenylhydantoin, the oxazolidines by trimethadione, and the succinimides by ethosuximide. Following short term administration of each agent, the microsomal demethylase activity of rat liver was determined. Dosages were approximately five times the recommended normal human daily dose for each of the agents (13).

MATERIALS AND METHODS

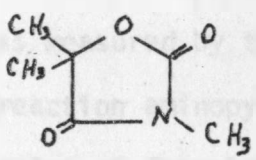
Adult, female Sprague-Dawley rats (3 months old, 159-225 grams) were used for this study. The animals were provided Purina Laboratory Chow and water ad libitum. The temperature of the room housing the animals was maintained at 75-78 degrees fahrenheit.

Compounds under investigation were all commonly used anti-epileptic agents. They included sodium phenobarbital (sodium 5-ethyl-5-phenylbarbiturate), sodium diphenylhydantoin (sodium 5,5-diphenyl-2,4-imidazolidinedione; Dilantin Sodium®, Parke-Davis), trimethadione (3,5,5-trimethyl-2,4-oxazolidinedione; Tridione®, Abbott) and ethosuximide (2-ethyl-2-methylsuccinimide; Zarontin®, Parke-Davis). Sodium phenobarbital was obtained from Merck and Company, Inc. of Rahway, New Jersey, and has the following chemical structure:



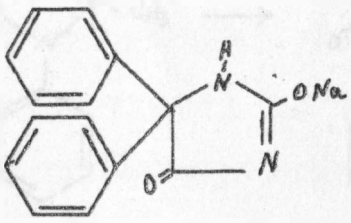
Sodium Phenobarbital

Trimethadione was obtained from Abbott Laboratories of North Chicago, Illinois, and has the following chemical structure:

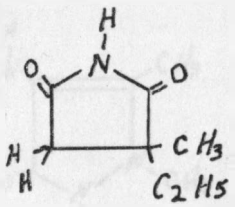


Trimethadione

Sodium diphenylhydantoin and ethosuximide were obtained from Parke, Davis and Company of Detroit, Michigan, and have the following chemical structures:



Sodium Diphenylhydantoin



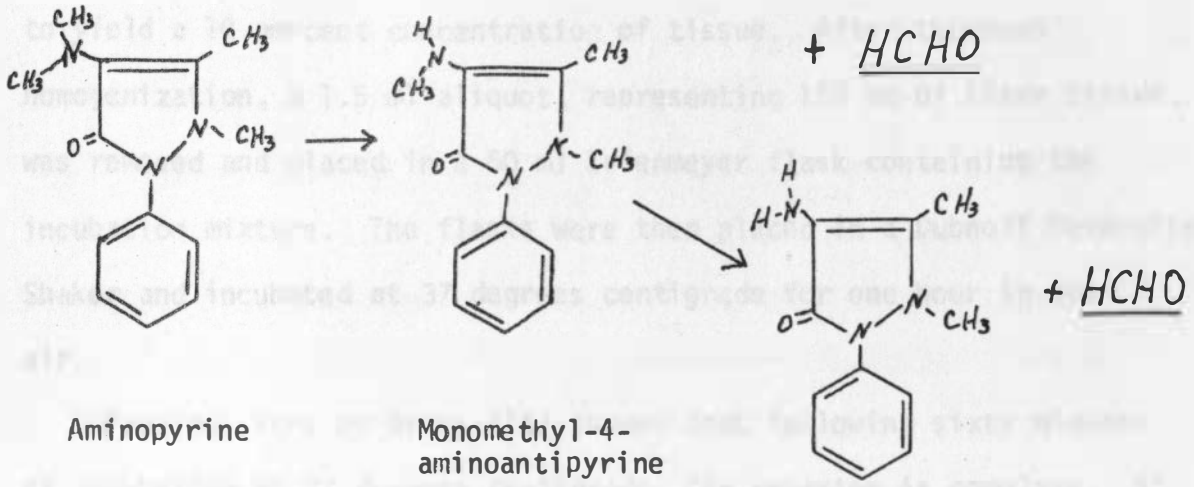
Ethosuximide

The drugs were prepared fresh daily in 0.9 per cent saline. Dosages employed were 9 mg/kg of sodium phenobarbital, 30 mg/kg of sodium diphenylhydantoin, 90 mg/kg of trimethadione and 54 mg/kg of ethosuximide. Dilutions of the drugs were prepared to allow administration of each compound at the rate of 3 ml/kg. All groups were given injections daily for five days prior to sacrifice. The dosages were calculated on the weight of the individual animals on the initial day of injection.

The animals were housed in pairs for approximately one month prior to testing. One day before the injections were to begin, they were randomly divided into groups of five animals, weighed and placed in individual cages. These cages were then placed in horizontal rows on the rack.

Demethylase activity was measured by the modified method of La Du et al. (7). In this reaction aminopyrine is demethylated by rat liver to yield two molecules of formaldehyde and one molecule of

4-aminoantipyrine. The following reaction illustrates the manner in which this demethylation occurs:



Aminopyrine

Monomethyl-4-aminoantipyrine

4-Aminoantipyrine

The addition of semicarbazide to the reaction mixture ties up the demethylated one carbon formaldehyde units and thus prevents them from escaping. The formaldehyde is released by the addition of 50 percent trichloroacetic acid solution. The quantity of formaldehyde can then be determined. These investigators also established many of the requirements for optimum demethylase activity. Each flask contained 100 micromoles of nicotinamide, 75 micromoles of magnesium chloride, 0.2 micromoles of nicotinamide adenine dinucleotide phosphate (NADP), 5 micromoles of aminopyrine, 2 micromoles of adenosine triphosphate, 3.8 micromoles of glucose-6-phosphate, 0.1 ml of 0.1 molar semicarbazide and phosphate buffer (pH 7.4) was added to bring the mixture to 5 ml.

On the sixth day following the initiation of the drugs, the rats were weighed and sacrificed by decapitation. A portion of the

liver was immediately removed, weighed and placed in a Potter Elvehjem glass homogenizer. Sufficient phosphate buffer (pH 7.4) was added to yield a 10 percent concentration of tissue. After thorough homogenization, a 1.5 ml aliquot, representing 150 mg of liver tissue, was removed and placed in a 50 ml Erlenmeyer flask containing the incubation mixture. The flasks were then placed in a Dubnoff Metabolic Shaker and incubated at 37 degrees centigrade for one hour in open air.

Previous work by Gross (14) showed that following sixty minutes of incubation at 37 degrees centigrade, the reaction is complete. At the conclusion of the incubation period, the flasks were removed from the incubator, cooled in an ice bath and the reaction stopped by the addition of 5 ml of 10 percent trichloroacetic acid solution. After swirling the flask to allow complete precipitation, the contents were centrifuged at 3700 X g for 10 minutes to cause separation of the protein material. Five ml of 50 percent trichloroacetic acid solution was then added resulting in a trichloroacetic acid concentration of approximately 20 percent. The purpose of this step was to free the formaldehyde from the semicarbazide so the volatile substance could then be distilled.

The supernatant was then placed in a conventional distillation apparatus and the first 5 ml of distillate was collected. From this 5 ml aliquot, a 1 ml portion was used for the formaldehyde assay.

Previous studies indicate that at least 99 percent of the formaldehyde present is distilled in the first 5 ml of distillate (14).

The chromotropic acid (4,5-dihydroxy-2,7-naphthalenedisulfonic acid disodium salt) method of Mac Fayden (15), modified by Mueller and Miller (16) and La Du et al. (7) was used to determine the amount of formaldehyde found. The amount of formaldehyde produced is directly proportional to the demethylation of aminopyrine; therefore, an accurate measurement of demethylase activity can be made. In this method a 1 ml aliquot of the distillate was added to a 4 ml portion of freshly prepared chromotropic acid solution. The mixture was then placed in a boiling water bath for 30 minutes. Caution was taken to minimize steam escaping around the tubes submerged in the water bath because of the hydroscopic nature of 12 molar sulfuric acid in the reagent. After a 30 minute development period, the tubes were removed, cooled, and the optical densities read in a Bausch and Lomb Spectronic 20 at 570 nanometers. The presence of formaldehyde was indicated by the development of a purple color.

The remainder of the liver was excised from the rat, rinsed in phosphate buffer (pH 7.4), pat dried and weighed. This weight plus the weight of the portion removed for the homogenate were combined to give the total rat liver weight.

The volume of original 10 percent tissue homogenate was sufficient to perform a protein determination. This determination was performed by the Biuret method (17). A 1 ml aliquot of the homogenate was diluted to 10 ml with distilled water. A 2 ml portion of this dilution was added

to 8 ml of Biuret reagent. Upon standing at room temperature for 30 minutes, the development of a blue color indicates the presence of protein in the solution. Because of a slight opacity due to undissolved material, all solutions were filtered prior to reading in a Bausch and Lomb Spectronic 20 at 550 nanometers. The extent of color development in the Biuret test is a quantitative indicator of the amount of protein present.

A standard curve for the Biuret test was established using four dilutions, 0.4, 0.6, 1.0, and 1.6 milligrams of protein. Pepsin was the protein used in the analysis. Four individual runs of each concentration were averaged to arrive at the standard curve (Figure 1). The results were read photometrically at 550 nanometers.

A standard curve for formaldehyde was made by using the following concentrations of formaldehyde: 0.1, 0.2, 0.3, 0.4, and 0.5 micromoles per 5 ml. These dilutions were then run through the chromotropic acid procedure and the data used to make the standard curve (Figure 2). This curve is the result of five determinations. The results were read photometrically at 570 nanometers.

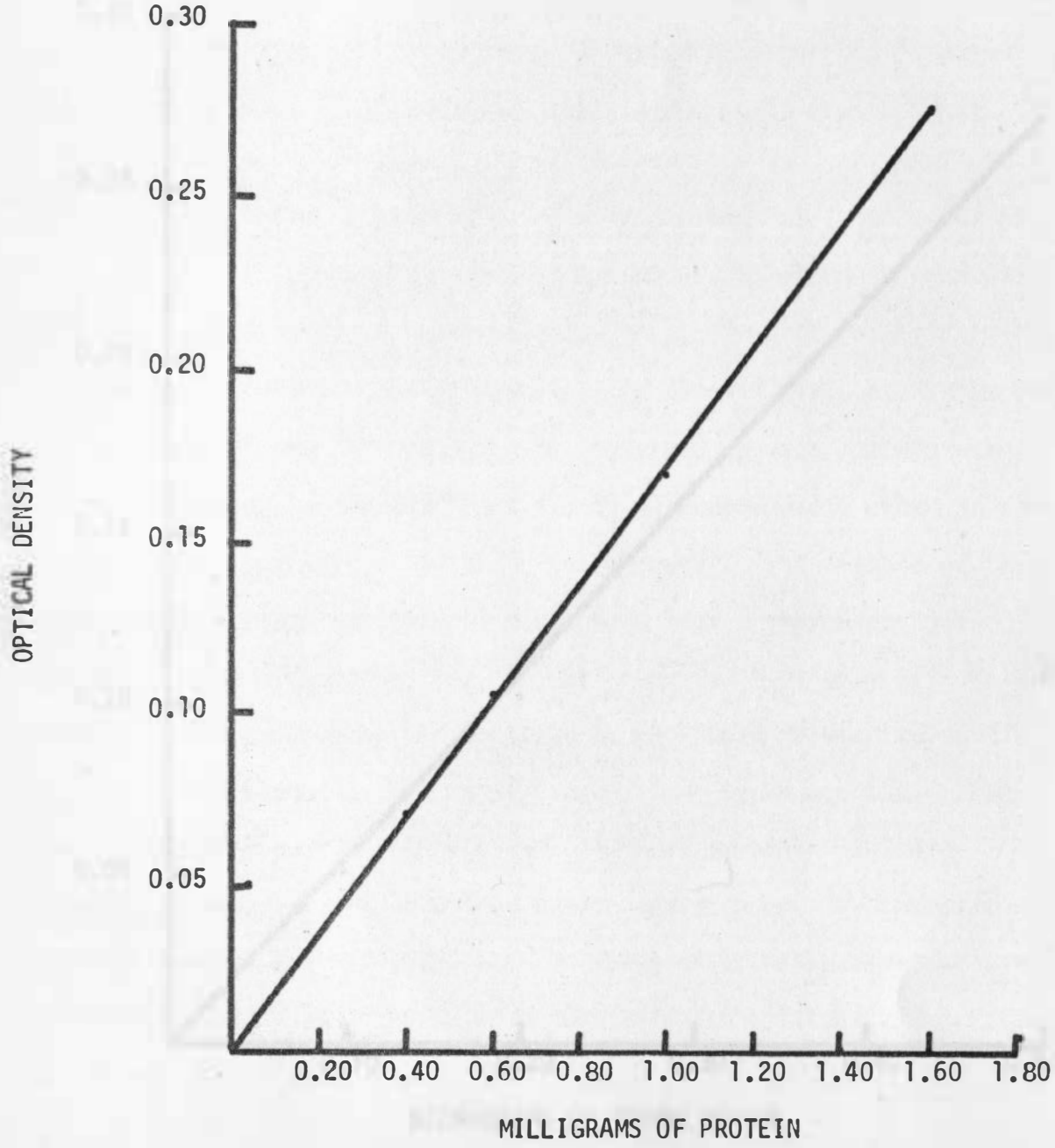


Fig. 1. Standard Biuret curve. Optical density versus milligrams of protein.

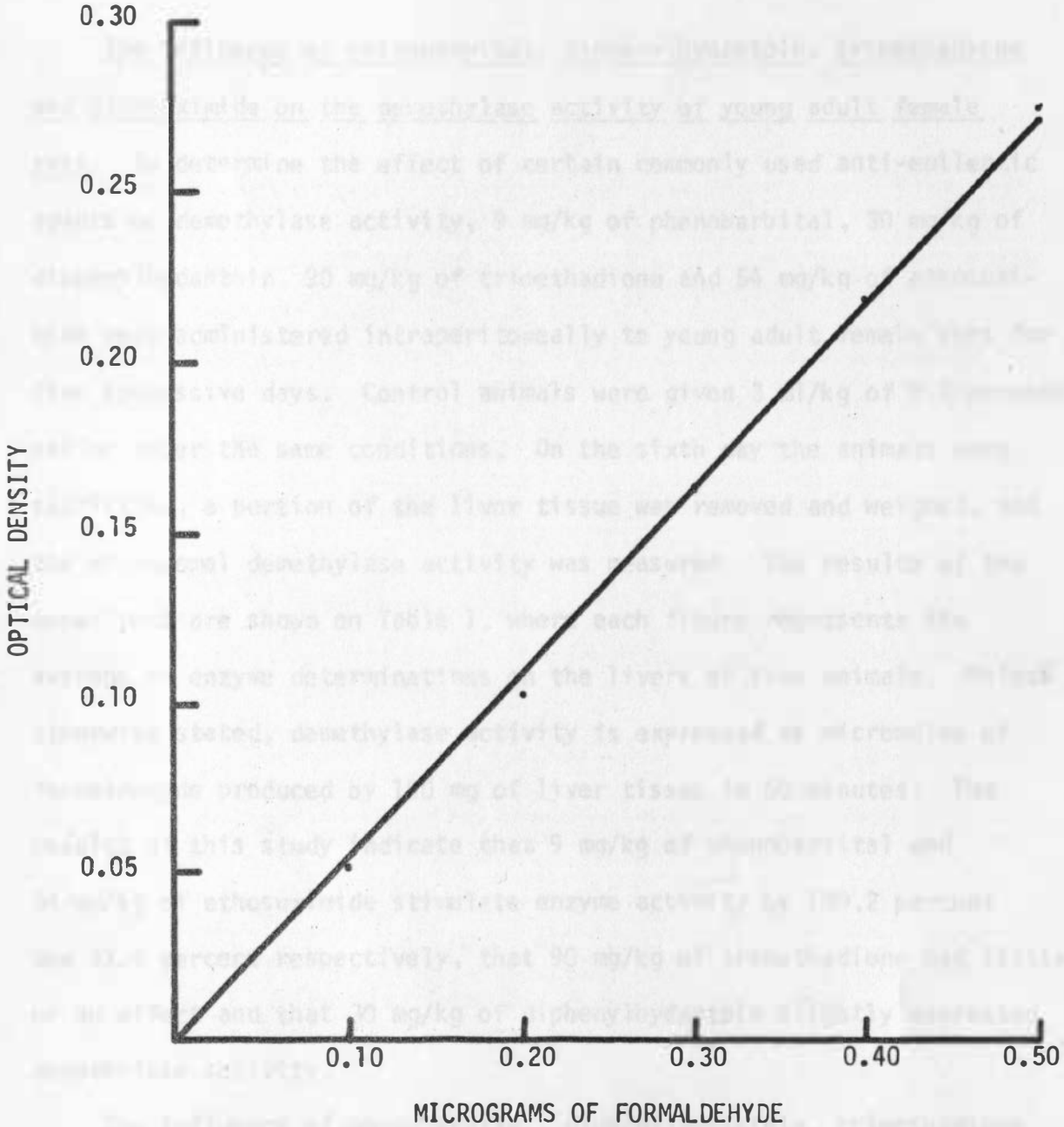


Fig. 2. Standard formaldehyde curve. Optical density versus micrograms of formaldehyde present.

RESULTS

The influence of phenobarbital, diphenylhydantoin, trimethadione and ethosuximide on the demethylase activity of young adult female rats. To determine the effect of certain commonly used anti-epileptic agents on demethylase activity, 9 mg/kg of phenobarbital, 30 mg/kg of diphenylhydantoin, 90 mg/kg of trimethadione and 54 mg/kg of ethosuximide were administered intraperitoneally to young adult female rats for five successive days. Control animals were given 3 ml/kg of 0.9 percent saline under the same conditions. On the sixth day the animals were sacrificed, a portion of the liver tissue was removed and weighed, and the microsomal demethylase activity was measured. The results of the experiment are shown on Table 1, where each figure represents the average of enzyme determinations on the livers of five animals. Unless otherwise stated, demethylase activity is expressed as micromoles of formaldehyde produced by 150 mg of liver tissue in 60 minutes. The results of this study indicate that 9 mg/kg of phenobarbital and 54 mg/kg of ethosuximide stimulate enzyme activity by 100.2 percent and 37.4 percent respectively, that 90 mg/kg of trimethadione had little or no effect and that 30 mg/kg of diphenylhydantoin slightly depressed demethylase activity.

The influence of phenobarbital, diphenylhydantoin, trimethadione and ethosuximide on the concentration of protein in rat liver tissue. To determine the effect of 9 mg/kg of phenobarbital, 30 mg/kg of diphenylhydantoin, 90 mg/kg of trimethadione and 54 mg/kg of ethosuximide on the protein concentration of rat liver tissue, 1 ml of the

TABLE 1

THE INFLUENCE OF PHENOBARBITAL, DIPHENYLHYDANTOIN, TRIMETHADIONE AND ETHOSUXIMIDE ON THE DEMETHYLASE ACTIVITY OF YOUNG ADULT FEMALE RATS

GROUP	ENZYME ACTIVITY* (Micromoles HCHO per 150 mg tissue per 60 minutes)	MICROMOLES HCHO ACTIVITY PER GRAM LIVER TISSUE*	PERCENT VARIATION**
Control	0.059 (0.045-0.072)	0.393	
Phenobarbital	0.118 (0.085-0.175)	0.787	+100.2
Diphenhylhydantoin	0.049 (0.047-0.053)	0.327	- 16.8
Trimethadione	0.060 (0.047-0.068)	0.400	+ 1.8
Ethosuximide	0.081 (0.049-0.113)	0.540	+ 37.4

* Represents the group average of five animals.
** Calculated on the micromoles of formaldehyde produced per gram of liver tissue.

original 10 percent liver tissue homogenate was diluted to 10 ml with distilled water. These dilutions were then analyzed for protein content. The results of the experiment are shown on Table 2, where each figure represents the average liver protein concentration of five animals. Data are expressed as milligrams of protein present per 100 milligrams of liver tissue. The results of this study indicate that 9 mg/kg of phenobarbital decreased liver protein concentration, that 90 mg/kg of trimethadione had little or no effect on liver protein concentration, and that 30 mg/kg of diphenylhydantoin and 54 mg/kg of ethosuximide increase liver protein concentration by 34.6 percent and 33.0 percent respectively.

The influence of phenobarbital, diphenylhydantoin, trimethadione and ethosuximide on body weight and liver weight of young adult female rats. To determine the effect of phenobarbital, diphenylhydantoin, trimethadione, and ethosuximide on total body weight of young adult female rats, each animal was weighed prior to drug administration and just before sacrifice. To determine the effect of these agents on liver weight, livers were excised, rinsed in phosphate buffer (pH 7.4), pat dried and weighed. The results of the experiment are shown on Table 3, where each figure represents the average value obtained from groups of five animals. Unless otherwise stated, data are expressed in grams of tissue. The results of this study indicate that the administration of 30 mg/kg of diphenylhydantoin caused a decrease in body weight; whereas 9 mg/kg of phenobarbital, 90 mg/kg of trimethadione and 54 mg/kg of ethosuximide caused a slight weight increase. Results

TABLE 2

THE INFLUENCE OF PHENOBARBITAL, DIPHENYLHYDANTOIN, TRIMETHADIONE AND ETHOSUXIMIDE ON THE CONTENT OF PROTEIN IN THE LIVER OF YOUNG ADULT FEMALE RATS

GROUP	LIVER TISSUE PROTEIN* (Mg per 100 mg liver tissue)	PERCENT VARIATION
Control	34.30 (32.00-38.00)	
Phenobarbital	27.25 (22.50-30.00)	-20.3
Diphenylhydantoin	46.05 (33.10-52.00)	+34.6
Trimethadione	33.95 (31.00-36.00)	- 0.7
Ethosuximide	45.50 (38.50-52.50)	+33.0

* Represents the group average of five animals.

TABLE 3

THE INFLUENCE OF PHENOBARBITAL, DIPHENYLHYDANTOIN, TRIMETHADIONE AND ETHOSUXIMIDE ON BODY WEIGHT AND LIVER WEIGHT OF YOUNG ADULT FEMALE RATS

GROUP	BEGINNING WEIGHT* (GM)	END WEIGHT* (GM)	NET CHANGE* (GM)	LIVER WEIGHT* (GM)	MG OF LIVER PER GM OF BODY WEIGHT*
Control	205.2	218.0	+12.8	6.864	31.49
Phenobarbital	197.6	207.8	+10.2	6.957	33.48
Diphenylhydantoin	209.6	185.6	-24.0	5.081	27.38
Trimethadione	180.2	208.6	+28.4	6.923	33.19
Ethosuximide	176.8	214.0	+37.2	7.129	33.31

* Represents the group average of five animals.

also indicate that the administration of 30 mg/kg of diphenylhydantoin caused a substantial decrease in the weight of the liver and that the injection of 9 mg/kg of phenobarbital, 30 mg/kg of trimethadione and 54 mg/kg of ethosuximide caused a slight increase in the weight of this group.

DISCUSSION

The purpose of this study was to obtain information concerning the effects of phenobarbital, diphenylhydantoin, trimethadione and ethosuximide on microsomal demethylase activity and liver tissue protein. In addition, the effects of these compounds on total body weight and liver weight were noted.

Barbiturates and hydantoins are used primarily for grand mal epilepsy; oxazolidines and succinimides are used primarily for the petit mal types (12). Members of more than one drug family may be used concurrently to control the epileptic patient, or one family may be substituted for another for the same purpose (10). It has not been established whether the demethylase enzyme system being investigated is specific for methyl groups or if it will dealkylate larger groups (7). The compounds employed in this study all contain alkyl groups in their chemical configuration. The influence of these agents on demethylase activity could be of value in situations where the physician is interchanging medications or attempting to arrive at a proper dosage regimen. Several investigators have noted that diphenylhydantoin levels are lowered in animals pre-treated and treated concurrently with phenobarbital (11,18).

In this study phenobarbital stimulated demethylase activity which concurs with previously published work (1-4). Although some investigators report greater degrees of induction (1,2), the dosages administered in their studies were considerably higher than those used in our experiments. A lower dosage was employed to more nearly

approach that which might be administered in a clinical situation. The dosages used were acquired by taking the usual adult daily dose of the drug in question and multiplying it by five (13). This quantity was divided by 70 (70 kilograms is considered the average adult weight) to obtain an average dose on a milligram per kilogram body weight basis. In our initial experiment, 9 mg/kg of phenobarbital, a compound generally considered to be the prototype of compounds which cause stimulation of various microsomal enzymes including demethylase, caused approximately a two-fold increase in demethylase activity; therefore, it was decided that this method of determining dosage and the research procedure were satisfactory for evaluation of other anti-epileptic agents.

Some disagreement exists in the literature concerning the effects of diphenylhydantoin on microsomal demethylase systems. Eling et al. (8) administered 100 mg/kg of diphenylhydantoin intraperitoneally and demonstrated an increase in demethylase activity; Platt and Cockrill (9) administered 200 mg/kg of diphenylhydantoin orally and found that this dosage did not effect the demethylase activity. In this study the intraperitoneal administration of 30 mg/kg of diphenylhydantoin caused a slight decrease in demethylase activity.

From the data acquired in this study, it was concluded that trimethadione caused no effect on demethylase activity. This conclusion parallels the findings of Conney (18).

The administration of 54 mg/kg of ethosuximide caused an appreciable increase in demethylase activity in the liver of the female rat. This

appears to be the first indication concerning the effects of succinimide compounds on microsomal enzyme systems.

Orrenius et al. (2) state that microsomal protein of the liver can be increased by prolonged treatment with phenobarbital. Arias et al. (3) report that phenobarbital does not alter protein degradation rates but rather increases protein synthesis in smooth endoplasmic reticulum. The results of this study indicate that 9 mg/kg of phenobarbital caused a decrease in the liver protein concentration and that 90 mg/kg of trimethadione caused no effect on the liver protein concentration.

The significance of the increased protein content of the group receiving 30 mg/kg diphenylhydantoin is clouded by the unusually small livers found in this group. Liver protein appeared to be increased by injections of 54 mg/kg of ethosuximide. Arias et al. (3) state that the half life of liver proteins is 2 to 2.5 days and a rapid turnover is continually taking place. Thus drug induced variations in liver protein would be possible with the 5-day treatment period.

Conney et al. (1) demonstrated both an increase in activity and an increase in actual liver size following phenobarbital administration. In our experiments the livers of rats treated with phenobarbital, trimethadione and ethosuximide were slightly larger than those of the control. Livers from diphenylhydantoin treated animals were somewhat smaller than the control as also was the body weight smaller than the control. Lesser values than control values occurred only in the latter group. The significance of these data is not understood.

Gilbert and Golberg state there is no correlation between liver size and the enzyme activity it may have (19). It has been reported that about one-third of the humans given large doses of diphenylhydantoin experience hyperlasia of the gums (20). If this manifestation should occur in rats, an explanation might then be available for the weight loss experienced by this group. Nothing was found in the literature discussing this reaction in rats. Literature on trimethadione (21) and ethosuximide (22) mention the possibility of drowsiness occurring while on these medications. Although the animals exhibited no outward signs of drowsiness, the occurrence is occasional and may influence body weight. In this study we did not monitor food and water consumption, or continuously observe the activity of the test animals. In order to more completely evaluate the effects of these compounds on microsomal enzyme activity, it would be necessary to obtain this information.

Observations during drug administration revealed the rats receiving diphenylhydantoin became groggy and lethargic post-injection. These effects were evident approximately 5 minutes after injections were made and persisted up to 120 minutes. The rats seemed mentally alert when handled yet when allowed to remain quiet, they preferred to lay down and remained listless throughout this period. The irritating effect of diphylhydantoin on the peritoneal cavity might explain this response (8).

SUMMARY

1. The modified procedure of La Du (13,14) was used to evaluate the microsomal enzyme system of the rat liver that demethylates aminopyrine.

2. The protein content of the liver tissue was measured by use of the standard Biuret test.

3. Daily intraperitoneal injections of 9 mg/kg of sodium phenobarbital to young adult female rats for five days caused approximately a two-fold increase in the demethylase activity of liver microsomes. The percentage of liver protein was decreased approximately 20 percent.

4. Daily intraperitoneal injections of 90 mg/kg of diphenylhydantoin to young adult female rats for five days caused a slight decrease in demethylase activity in the liver microsomes. Liver protein was considerably elevated following drug administration.

5. Daily intraperitoneal injections of 30 mg/kg of trimethadione to young adult female rats for five days caused no variation in the demethylase activity in liver microsomes. The Biuret test showed that this treatment did not effect the protein concentration of the liver.

6. Daily intraperitoneal injections of 54 mg/kg of ethosuximide to young adult female rats for five days caused an appreciable increase in the demethylase activity of the liver microsomes. Protein analysis indicated that this treatment caused approximately 33 percent increase in protein content of the liver.

REFERENCES

1. Conney, A., Davison, C., Gastel, R., and Burns, J., J. Pharmacol. Exptl. Therap., 130: 1-8 (1960).

2. Orrenius, S., Manik, D., and Gnosspelius, P., in Microsomes and Drug Oxidations, Academic Press, New York, p. 251 (1969).

3. Arias, I., Doyle, D., and Schimke, R., in Microsomes and Drug Oxidations, Academic Press, New York, p. 453 (1969).

4. Holtzman, J., and Gillette, J., Biochem. Biophys. Res. Com., 24: 639 (1966).

5. Conney, A., and Gilman, A., J. Biol. Chem., 238: 3682 (1963).

6. Orrenius, S., Ericsson, J., and Ernster, L., J. Cell. Biol., 25: 627 (1965).

7. La Du, B., Gaudette, L., Trousof, N., and Brodie, B., J. Biol. Chem., 214: 741 (1955).

8. Eling, T., Harbison, R., Becker, B., and Fouts, J., J. Pharmacol. Exptl. Therap., 171: 127 (1970).

9. Platt, D., and Cockril, B., Biochem. Pharmacol., 18: 429 (1969).

10. Goodman, L., and Gilman, A., in The Pharmacological Basis of Therapeutics, 3rd Ed., MacMillan Co., New York, p. 219 (1965).

11. Cucinell, S., Koster, R., Conney, A., and Burns, J., J. Pharmacol. Exptl. Therap., 141: 157 (1963).

12. Goth, A., in Medical Pharmacology, 4th Ed., Mosby Co., St. Louis, p. 301 (1968).

13. Goodman, L., and Gilman, A., in the Pharmacological Basis of Therapeutics, 3rd Ed., MacMillan Co., New York, p. 222, 225, 230, 233 (1965).

14. Gross, G., The Effect of Norethandrolone of the Liver Microsomal Enzyme Which Demethylates Aminopyrine, M.S. Thesis, SDSU (1967).

15. Mac Fayden, D., J. Biol. Chem., 158: 107 (1945).

16. Mueller, G., and Miller, J., J. Biol. Chem., 202: 579 (1953).

17. Clark, J., in Experimental Biochemistry, Freeman and Co., San Francisco, p. 75, 95, 217 (1964).

18. Conney, A., Pharmacol. Rev., 19: 317 (1966).

19. Gilbert, D., and Golberg, L., Fd. Cosmet. Toxicol., 3: 417 (1965).

20. Goth, A., in Medical Pharmacology, 4th Ed., Mosby Co., St. Louis, p. 303 (1968).

21. Tridione Package Literature, Abbott Laboratories, North Chicago, Illinois.

22. Zarontin Package Literature, Parke, Davis and Co., Detroit, Michigan.